

Classification of the Spore-Forming Sulfate-Reducing Bacteria

L. LEON CAMPBELL AND JOHN R. POSTGATE

Department of Microbiology, University of Illinois, Urbana, Illinois, and A.R.C. Unit of Nitrogen Fixation, University of Sussex, Falmer, Sussex, England

INTRODUCTION.....	359
REASONS FOR SEPARATING SPORULATING FROM NONSPORULATING SULFATE-REDUCING BACTERIA.....	359
COMMENTS ON THE SEPARATION PROPOSED.....	360
NOMENCLATURE OF THE SPORE-FORMING TYPES.....	360
NOMENCLATURE OF <i>DESULFOVIBRIO ORIENTIS</i>	361
NOMENCLATURE OF COLEMAN'S ORGANISM.....	361
DESCRIPTION OF THE GENUS <i>DESULFOTOMACULUM</i> (CAMPBELL AND POSTGATE).....	361
<i>Desulfotomaculum nigrificans</i>	361
<i>Desulfotomaculum ruminis</i>	361
<i>Desulfotomaculum orientis</i>	361
INTERPRETATION OF EARLIER WORK.....	362
LITERATURE CITED.....	362

INTRODUCTION

Early work by Kluyver and Baars (3, 11) and Starkey (26), together with verbal support from Bunker (6) and K. R. Butlin's research group (7, 16), supported the view that mesophilic, monotrichous, nonsporulating, sulfate-reducing bacteria could be converted into thermophilic, peritrichous, sporulating organisms by "training," and that the reverse process could also be induced. Pochon and Chavignac (14) described a comparable change, a loss of capacity to sporulate, on prolonged laboratory culture of a mesophilic strain. With the identification of the thermophilic, sporulating sulfate-reducer as *Clostridium nigrificans* by Campbell, Frank, and Hall (8), doubt about the interconvertibility of the two types arose, and subsequent work (18) has tended to reinforce these doubts. A need for rationalization of the nomenclature of the sulfate-reducing bacteria in the light of work of the last decade is agreed to by most workers in the field. In this publication we propose a revised classification for the sporulating sulfate-reducing bacteria.

REASONS FOR SEPARATING SPORULATING FROM NONSPORULATING SULFATE-REDUCING BACTERIA

Two mesophilic, spore-forming, peritrichous species of sulfate-reducing bacteria, *Desulfovibrio orientis* (1) and Coleman's organism (9), have been isolated; they are obligate mesophiles.

The nonsporulating, monotrichous vibrios have been shown to have characteristic cell pigments (cytochrome c_3 , and desulfoviridin) which are

uniformly absent from the sporulating types (17, 18).

The mesophilic isolates obtained from heated soils, which must be presumed to sporulate, have been shown not to have the pigment desulfoviridin (2) and must therefore resemble Coleman's organism or *D. orientis*.

The sporulating mesophiles have some antigenic relationship to the sporulating thermophiles, that of Coleman's organism being close, and that of *D. orientis* being slight but observable (20). None of these types shows cross antigenicity with the nonsporulating mesophiles (8, 20); cross antigenicity among various types of nonsporulating mesophiles occurs (4, 8), and extends even to obviously distinct species such as *D. gigas* (12).

The base composition of deoxyribonucleic acid (DNA) from *C. nigrificans* is remote from that of most nonsporulating types (23, 24, 25) and is close to that of the two mesophilic spore-formers (20, 23).

H. J. Bunker and K. R. Butlin kindly made their experimental records available to one of us (J. R. P.); on careful examination, neither provided proof that the mesophilic, monotrichous type, and the thermophilic, peritrichous type, were interconvertible. Their experiments provided no reason to doubt the then-current acceptance of this phenomenon. This question is discussed below.

In a sequence of experiments initiated before Campbell, Frank, and Hall's (8) identification of the thermophile as *C. nigrificans* was published, one of us (J. R. P.) trained seven thermophilic strains to mesophily and attempted to train five

mesophiles to thermophily. No mesophile could be acclimatized to growth above 50 C; no *permanent* morphological change took place in any strain, though transient aberrants resembling those described by Starkey (26), and by Butlin et al. (7), were observed. These experiments have been mentioned (18) but not in detail; they support the findings of Campbell et al. (8). The antigenic relationship of *C. nigrificans* to the thermophilic sulfate-reducer was also independently verified.

Examination of the morphology of many strains has led us to the conclusion that the spore-forming and nonsporulating types show consistent differences. Cells of the sporulating species, with the exception of *D. orientis*, are ordinarily straight (1, 5, 20); such aberrants as appear, even in conditions of stress, are lenticulate, prespore, or irregular filamentous forms. Cells of the nonsporulating mesophiles are, with the exception of certain nitrogen-fixing strains, curved, and their aberrants tend to be sigmoid or spirilloid in shape. When they are motile, cells of the spore-formers show a characteristic "twisting and turning" or oscillatory motility (8, 20); the nonsporulating types show rapid progressive motility often accompanied by a rapid "wobble" (18).

Considerable effort has been devoted by ourselves and other workers to find a desulfoviridin-positive, sulfate-reducing organism which is thermophilic. None has so far been obtained; this implies that all thermophilic sulfate-reducing bacteria are of the desulfoviridin-free group which resembles *C. nigrificans*. These findings, taken together, lead to the view that the sporulating sulfate-reducing bacteria are physiologically and morphologically related, but that they are unrelated to the nonsporulating types.

COMMENTS ON THE SEPARATION PROPOSED

The base composition of DNA from one group of nonsporulating, monotrichous, mesophilic, sulfate-reducing vibrios, which possess cytochrome *c*₃ and desulfoviridin, is identical with that of one of the mesophilic spore-formers: group "3" of Saunders et al. (23) and Coleman's organism have nearly identical values. The other properties clearly separate these two groups, and it is legitimate to regard the DNA base composition overlap as coincidental.

A nonsporulating mesophilic strain has been isolated which does not contain desulfoviridin, but which is otherwise like most others, even to the extent of containing cytochrome *c*₃ [strain Norway 4 (13)]. This strain is unique among a great many tested in various laboratories, and its existence does not reduce the weight of the de-

sulfoviridin fluorescence test as a taxonomic criterion (17).

Prévot (22) maintained that, despite the report of Campbell et al. (8), both *C. nigrificans* and *Sporovibrio thermodesulfuricans* exist as separate species, and that sulfate reduction is a property of certain *Clostridium* species that is lost rapidly on laboratory subculture. Our proposal excludes from the genus *Desulfotomaculum* mesophilic clostridia which show transient sulfate reduction (21).

NOMENCLATURE OF THE SPORE-FORMING TYPES

If the spore-forming sulfate-reducing bacteria are separated from the nonsporulating types, the specific epithet *desulfuricans* must remain with the nonsporulating group, and so must the generic name *Desulfovibrio*, since the latter is the present equivalent of Beijerinck's *Spirillum*. The name *Clostridium nigrificans* (27) was given nomenclatural priority by Campbell et al. (8), but not only would it be incorrect to transfer *Desulfovibrio orientis* and Coleman's organism to the genus *Clostridium*, but also we believe that the classification by Werkman and Weaver (27) of the original *nigrificans* in the genus *Clostridium* was a mistake for these reasons: (i) Most clostridia are gram-positive. All strains of *C. nigrificans* that we have examined are gram-negative. (ii) The DNA base composition of *C. nigrificans* (23, 24, 25) is remote from that of the few typical clostridia so far examined (24). (iii) The presence of cytochromes in the three sporulating types (20) is not typical of clostridia.

For these reasons we propose that *C. nigrificans* be removed from the genus *Clostridium* and be reclassified with the mesophilic spore-formers under a new generic name. While recognizing the *International Code* recommendation to "avoid names that are long or difficult to pronounce," we feel that the prefix "Desulfo" should be retained to indicate that dissimilatory sulfate reduction is a fundamental taxonomic property. We exclude the designation *Sporovibrio* because the vibrio form is not typical of cells of this group, and because we wish to avoid confusion with unrelated organisms such as *Sporovibrio ferrooxydans* (15). Since "-bacterium," "-bacillus," and "-clostridium" are excluded because our group forms spores, is not aerobic, and is gram-negative, we propose to signify that the cells of species of this genus can be straight, curved, or swollen by introducing the Latin suffix *tomaculum* (or *tomachum*), meaning sausage. Hence, we propose the generic name *Desulfotomaculum*, with *Desulfotomaculum nigrificans* as the type species. In the absence of Werkman's original strain, we propose

Starkey's strain Delft 74T (N.C.I.B. 8395) as the neotype of this species.

NOMENCLATURE OF *DESULFOVIBRIO ORIENTIS*

This species is readily translated into *Desulfotomaculum orientis*, with strain Singapore I (N.C.I.B. 8382) as the holotype species.

NOMENCLATURE OF COLEMAN'S ORGANISM

Coleman (9) did not name his strain. Postgate and Campbell (20) noted that it possessed two apparently stable characteristics different from those of its thermophilic relative: its obligate mesophily, and its ability to utilize formate. The latter character is of uncertain taxonomic weight but coupled with the former may justify distinct speciation. Therefore we propose the name *Desulfotomaculum ruminis*, the specific epithet referring to the source of Coleman's culture, with strain Coleman 42 (N.C.I.B. 8452) as holotype. G. S. Coleman has authorized us to make such a proposal.

DESCRIPTION OF THE GENUS *DESULFOTOMACULUM* (CAMPBELL AND POSTGATE)

From M.L. *desulfo*, used to indicate reduction of sulfur compounds by bacteria; *L. tomaculum* (*tomaclum*), sausage.

Gram-negative, straight or curved rods, usually single but sometimes in chains; thermophilic types often show lenticulate and otherwise swollen forms. Terminal or subterminal sporulation, slightly swelling the cells. Motile, with peritrichous flagella. Obligate anaerobes which reduce sulfate to sulfide; species show cross antigenicity by agglutination and immunodiffusion tests. Found in fresh water, soils, geothermal regions, certain spoiled foods, intestines of insects, and in rumen contents of ruminant mammals.

The type species is *Desulfotomaculum nigrificans* (Werkman and Weaver) Campbell and Postgate.

1. ***Desulfotomaculum nigrificans*** (Werkman and Weaver) Campbell and Postgate.

[*Vibrio thermodesulfuricans* Elion (10); *Sporovibrio desulfuricans* Starkey (26); *Clostridium nigrificans* Werkman and Weaver (27).] From *L. niger*, black, and *faciens*, making.

Rods, 0.3 to 0.5 by 3 to 6 μ , rounded ends, sometimes lenticulate and swollen, sometimes paired. Motile with "twisting and tumbling" motility, peritrichous flagella. Spores oval, terminal or subterminal, slightly swelling the cells. Gram-negative.

Gelatin: not liquefied.

Deep agar colonies: black, particularly if ferrous salts present.

Nitrate: not reduced.

Glucose and other carbohydrates: not fermented.

Coagulated albumin: not liquefied.

Blood serum: not liquefied.

Grows in specialized media (8, 20) containing lactate or pyruvate (but not formate or acetate) plus sulfate, and produces H_2S . Grows without sulfate if pyruvate is present (19).

H_2S formed from cystine.

No reported pathogenicity to man, guinea pig, mouse, rat, or rabbit.

Thermophilic: optimal temperature, 55 C; can grow at 65 to 70 C. Can be "trained" to grow slowly at 37 or 30 C.

DNA: 44.5% guanine + cytosine.

Cytochrome: of the protohem class.

Anaerobic.

Habitat: soils, compost heaps, thermal spring water, "sulfur stinker" spoiled foods.

2. ***Desulfotomaculum ruminis*** (Coleman) Campbell and Postgate. From *L. rumen*, throat, adopted for first stomach of a ruminant.

Rods, 0.5 by 3 to 6 μ ; rounded ends, sometimes paired. Slight tumbling motility, peritrichous flagella. Spores oval, terminal or subterminal, slightly swelling the cells. Gram-negative.

Deep agar colonies: black, particularly if iron salts are present.

Nitrate: not reduced.

Glucose and other carbohydrates: not fermented.

Coagulated albumin, blood serum: not liquefied.

Grows on specialized media (20) containing lactate, pyruvate, or formate (but not acetate) plus sulfate, and produces H_2S . Grows without sulfate if pyruvate is present.

No recorded pathogenicity to man, guinea pig, mouse, rat, or rabbit.

Mesophilic: optimal temperature, 37 C; upper limit, 48 C.

DNA: 45.5% guanine + cytosine.

Cytochrome: of the protohem class.

Anaerobic.

Habitat: rumen contents of sheep.

3. ***Desulfotomaculum orientis*** (Adams and Postgate) Campbell and Postgate.

[*Desulfovibrio orientis*, Adams and Postgate (1).] From *L. oriens*, the East.

Fat curved rods, 1.5 by 5 μ , sometimes paired. "Tumbling and twisting" motility, peritrichous flagella. Spores round, central, paracentral or terminal, slightly swelling the cells. Gram-negative.

Deep agar colonies: black, particularly if iron salts are present.

Gelatin: not hydrolyzed.

Nitrate: not reduced.

Glucose and other carbohydrates: not fermented.

Grows in specialized media (1, 20) containing lactate or pyruvate (but not formate or acetate) plus sulfate and thioglycolate, and produces H_2S . Does not grow without sulfate, even with pyruvate.

No recorded pathogenicity to man, guinea pig, mouse, rat, or rabbit.

Mesophilic: optimal temperature, between 30 and 37 C; upper limit, 42 C.

DNA: 41.7% guanine + cytosine.

Cytochrome: of the protohem class.

Anaerobic.

Habitat: isolated from soil from Singapore.

INTERPRETATION OF EARLIER WORK

Much of the early work on the interconvertibility of sporulating and nonsporulating types can be interpreted on the assumption that the initial populations, although pure with respect to nonsulfate-reducing bacteria, contained representatives of both *Desulfovibrio* and *Desulfotomaculum* species. On the question of the change in flagellation, both types of bacteria have fragile flagella, and, from certain of the experimental data made available to us, it was clear that most of the cells of the isolates studied had lost their flagella during preparation for electron microscopy. On the question of adaptation to thermophily, in at least one instance error was made by not preheating the medium to the temperature of the incubator, so that growth and production of compounds responsible for blackening which had taken place while the culture was warming up was accepted as growth at the recorded temperature of the incubator. In that case the viability of the blackened culture was not checked. In other cases the experimental records provide no such simple explanation.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant AI-04673 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

- ADAMS, M. E., AND J. R. POSTGATE. 1959. A new sulphate-reducing vibrio. J. Gen. Microbiol. **20**:252-257.
- ADAMS, M. E., AND J. R. POSTGATE. 1961. On sporulation in sulphate-reducing bacteria. J. Gen. Microbiol. **24**:291-294.
- BAARS, J. K. 1930. Over sulfaatreductie door bacterien. Thesis, Meirema, Delft, The Netherlands.
- BAKER, F. D., H. R. PAPISKA, AND L. L. CAMPBELL. 1962. Choline fermentation by *Desulfovibrio desulfuricans*. J. Bacteriol. **84**:973-978.
- BULLER, C. S., AND J. M. AKAGI. 1964. Hydrogenase of Coleman's sulfate-reducing bacterium. J. Bacteriol. **88**:440-443.
- BUNKER, H. J. 1953. Discussion. In Adaptation in microorganisms. Symp. Soc. Gen. Microbiol., p. 324. Cambridge Univ. Press.
- BUTLIN, K. R., M. E. ADAMS, AND M. THOMAS. 1949. The morphology of sulphate-reducing bacteria. J. Gen. Microbiol. **3**:iii.
- CAMPBELL, L. L., JR., H. A. FRANK, AND E. R. HALL. 1957. Studies on thermophilic sulfate reducing bacteria. I. Identification of *Sporovibrio desulfuricans* as *Clostridium nigrificans*. J. Bacteriol. **73**:516-521.
- COLEMAN, G. S. 1960. A sulphate-reducing bacterium from the sheep rumen. J. Gen. Microbiol. **22**:423-436.
- ELION, L. 1924. A thermophilic sulphate-reducing bacterium. Zentr. Bakteriell. Parasitenk. Abt. II **63**: 58-67.
- KLUYVER, A. J., AND J. K. BAARS. 1932. On some physiological artefacts. Proc. Koninkl. Akad. Westenchap. Amsterdam **35**:370-378.
- LE GALL, J. 1963. A new species of *Desulfovibrio*. J. Bacteriol. **86**:1120.
- MILLER, J. D. A., AND A. M. SALEH. 1964. A sulphate-reducing bacterium containing cytochrome C_3 : but lacking desulfovibrin. J. Gen. Microbiol. **37**:419-423.
- POCHON, J., AND M. CHALVIGNAC. 1952. Sur l'instabilité des caractères d'une souche de *Sporovibrio*. Ann. Inst. Pasteur **82**:399-407.
- POCHON, J., AND H. DE BARJAC. 1954. Une espèce nouvelle de *Sporovibrio*: *S. ferrooxydans* (N. Sp.). Compt. Rend. **238**:627.
- POSTGATE, J. R. 1953. Discussion. In Adaptation in microorganisms. Symp. Soc. Gen. Microbiol., p. 324. Cambridge Univ. Press.
- POSTGATE, J. R. 1959. A diagnostic reaction of *Desulphovibrio desulphuricans*. Nature **183**:481.
- POSTGATE, J. R. 1959. Sulphate reduction by bacteria. Ann. Rev. Microbiol. **13**:505-520.
- POSTGATE, J. R. 1963. Sulfate-free growth of *Clostridium nigrificans*. J. Bacteriol. **85**:1450-1451.
- POSTGATE, J. R., AND L. L. CAMPBELL. 1963. Identification of Coleman's sulfate-reducing bacterium as a mesophilic relative of *Clostridium nigrificans*. J. Bacteriol. **86**:274-279.
- PRÉVOT, A-R. 1948. Recherche sur la réduction des sulfates et des sulfites minéraux par les bactéries anaérobies. Ann. Inst. Pasteur **75**:571-572.
- PRÉVOT, A-R. 1958. Manuel de classification et de détermination des bactéries anaérobies, 3rd ed. Masson et Cie., Paris.
- SAUNDERS, G. F., L. L. CAMPBELL, AND J. R. POSTGATE. 1964. Base composition of deoxy-

- ribonucleic acid of sulfate-reducing bacteria deduced from buoyant density measurements in cesium chloride. *J. Bacteriol.* **87**: 1073-1078.
24. SCHILDKRAUT, C. L., J. MARMUR, AND P. DOTY. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. Mol. Biol.* **4**:430-443.
25. SIGAL, N., J. C. SENEZ, J. LE GALL, AND M. SEBALD. 1963. Base composition of the deoxyribonucleic acid of sulfate-reducing bacteria. *J. Bacteriol.* **85**:1315-1318.
26. STARKEY, R. L. 1938. A study of spore formation and other morphological characteristics of *Vibrio desulfuricans*. *Arch. Mikrobiol.* **9**:368-404.
27. WERKMAN, C. H., AND H. J. WEAVER. 1927. Studies in the bacteriology of sulphur stinker spoilage of canned sweet corn. *Iowa State Coll. J. Sci.* **2**:57-67.